

CLAIMS

We claim:

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1. A purified preparation of primate embryonic stem cells which (i) is capable of proliferation in vitro culture for over one year, (ii) maintains a normal karyotype through prolonged culture, (iii) maintains the potential to differentiate to derivatives of endoderm, mesoderm, and ectoderm tissues throughout the culture, and (iv) will differentiate in the presence of human leukemia inhibitory factor alone, but will not differentiate when cultured on a fibroblast feeder layer.

2. The preparation of claim 1 wherein the stem cells will spontaneously differentiate to trophoblast and produce chorionic gonadotropin when cultured to high density.

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3. A purified preparation of primate embryonic stem cells wherein the cells are negative for the SSEA-1 marker, positive for the SSEA-3 marker, positive for the SSEA-4 marker, express alkaline phosphatase activity, are pluripotent, and have normal karyotypes.

4. The preparation of claim 3 wherein the cells are positive for the TRA-1-60, and TRA-1-81 markers.

5. The preparation of claim 3 wherein the cells continue to proliferate in an undifferentiated state after continuous culture for at least one year.

6. The preparation of claim 3 wherein the cells will differentiate to trophoblast when cultured beyond confluence and will produce chorionic gonadotropin.

7. The preparation of claim 3 wherein the cells remain euploid for more than one year of continuous culture.

5 8. The preparation of claim 3 wherein the cells differentiate into cells derived from mesoderm, endoderm and ectoderm germ layers when the cells are injected into a SCID mouse.

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9. A method of isolating a primate embryonic stem cell line, comprising the steps of:

- (a) isolating a primate blastocyst;
- (b) isolating cells from the inner cell mass of the blastocyte of (a);
- (c) plating the inner cell mass cells on embryonic fibroblasts, wherein inner cell mass-derived
- 15 cell masses are formed;
- (d) dissociating the mass into dissociated cells;
- (e) replating the dissociated cells on embryonic feeder cells;
- 20 (f) selecting colonies with compact morphologies and cells with high nucleus to cytoplasm ratios and prominent nucleoli; and
- (g) culturing the cells of the selected colonies.

25 10. A method as claimed in claim 9 further comprising maintaining the isolated cells or a fibroblast feeder layer to prevent differentiation.

11. A cell line developed by the method of step 9.